Haemostatic Issues in Cancer Development and Progression:

"The Role of Coagulation and Haemostatic Factors in cancer Development and Metastasis"

Ph.D. thesis

(Summary)

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Introduction: Thrombosis is a well-recognized and common complication in patients with malignant disease and can contribute significantly to the morbidity and mortality of this disease. The occurrence of thrombosis is heightened by therapeutic interventions such as operations or the use of radio-chemotherapy. It occurs both spontaneously, after surgery, after radiation therapy and in medical cancer patients receiving anti-cancer treatment. It may also be the first manifestation of underlying malignant disease. The magnitude of the risk for venous thromboembolism is well established for cancer surgery where rates twice that for abdominal surgery in non-cancer patients are described. Venous thromboembolism is the most common complication of cancer and the second most common cause of death in cancer patients. Up to 60% of patients with cancer develop venous thromboembolism, depending on the type of cancer and the treatment given. Although the close relationship between tumour growth and the activation of blood coagulation has been known since 1865, when Professor Armand Trousseau first described the clinical association between primary or idiopathic venous thromboembolism and occult malignancy, only in the last two decade have significant advances in this field been achieved.

It is now well known that the clinical manifestation of thrombosis in patients with cancer can be very different and vary from localized venous thromboembolism to disseminated intravascular coagulation. In addition, a sub-clinical activation of blood coagulation or "hypercoagulable state" is present in almost all cancer patients, even without symptoms of thrombosis. A number of pathogenetic factors have been identified, showing that activation of coagulation in cancer is a complex phenomenon, involving many different pathways of the haemostatic system and numerous interactions of the tumour cells with other blood cells, including platelets, monocytes and endothelial cells.

In this thesis, the pathogenetic mechanisms of thrombosis in malignancy, thrombophilic state in cancer patients, changes in haemostatic parameters and their relation to cancer prognosis and also the thromboprophylaxis in the cancer patients are discussed and analyzed.

Aim of Study: The aim of this prospective study is to evaluate the changes in haemostatic-clotting parameters in patients with different types of non-haematological malignancies (solid tumours) and to asses the correlation between changes in coagulation parameters and the stage of tumour, imaging findings and also with changes in characteristic tumour markers. In our prospective study, we mainly focused on specific abnormalities of haemostasis in these groups of patients, the changes in haemostatic parameters and their relation to cancer prognosis. In addition, the thromboprophylaxis in the cancer patients are discussed and analyzed.

This study also provides a rationale for the use anticoagulants for the prevention of thromboembolic complications and may change the course of tumour progression.

Inclusion in the study required tissue diagnosis for histopathology classification, detailed imaging techniques for exact staging, and absence of any medications that might interfere with the results of hypercoagulation markers. The study design and outcomes evaluations mirrored those used in prior studies, except that our study used more than one coagulation parameter for a more detailed assessment of the changes in haemostatic system in cancer patients.

Except for a slight difference in the gender ratio, the analysis of patients who met the pre-specified criteria for evaluation, the hypercoagulable parameters were directly correlated with tumour progression and rise in characteristic tumour markers.

Parallel with the haemostatic parameters, the characteristic tumour markers were also measured. In all studied cases, there was a direct correlation between changes in the haemostatic parameters, tumour markers and radiological-imaging findings, e.g.: rise in D-dimer, F 1+2 was associated with a drop in AT, PS, PC, which was directly correlated with a rise in tumour markers and a progression of the malignant diseases in imaging findings.

The study design and evaluations mirrored those used in prior studies, except that in our study we used more than one coagulation parameter for a more detailed assessment of the changes in haemostatic system in this group of cancer patients. The patients were enrolled in this study prior to any type of treatment. Additional criteria for enrolment were absence of previous thromboembolic event in the past 12 months, absence of any heparin derivatives, oral anticoagulant agents and also anti-platelet drugs. Patients with

suspected distant metastasis were excluded. Patients who received any type of anticoagulant or hormonal treatment in the past 6 months were also excluded. Patients having abnormal kidney and liver function tests were also not enrolled.

Fifty-four patients were initially enrolled in this study;

2 had oesophageal adenocarcinoma, 6 had gastric cancer, 16 had colorectal cancer, 4 had exocrine pancreatic carcinoma, 2 had adenocarcinoma of gallbladder, 6 had adenocarcinomatous type of non-small cell lung cancer, and 2 had small cell lung cancer,

12 had infiltrating breast carcinoma, and 2 had ovarian cancer. Four patients were excluded because they developed VTE during the period of study. Detailed monitoring upon admission and prior to any cancer-related intervention and on a weekly basis post-intervention (chemotherapy, radiation or surgery) for up 18 weeks were carried out. The most important natural inhibitors of abnormal coagulation (PC, PS, and AT), and D-dimer and prothrombin activation peptide F 1 +2 as markers of the status of fibrinolytic and coagulation systems were studied in these group of patients prior to any form of therapy. These markers were repeatedly measured with each treatment course and their results were correlated with other markers of tumour prognosis

Statistical Analysis: The statistical analysis was performed by two-way analysis of variance (ANOVA) comparing the markers of haemostasis activation at admission to post-admission for each subject and with respect to average control values; differences were considered significant at p value of 0.05 or less. The Cox proportional hazards regression model was used to adjust the treatment effect on survival for baseline factors in all patients with solid tumours, and for the subgroups with and without metastasis. The variables identified as potentially important predictors, and recorded at the time of enrolment, included age, gender, ECOG performance status, smoking status (ever vs. never), type of cancer treatment (radiation vs. none, chemotherapy vs. none), and major primary site (breast, lung, colorectal, pancreas, and gynaecologic). D-dimer, prothrombin fragment 1 + 2 (F1 + 2), antithrombin, protein C, and protein S activities were also measured at the onset of diagnosis, pre- and post surgery, and after the completion of each chemotherapy course. Their levels were correlated with the levels of tumour markers.

Findings and Discussion: The changes in clotting parameters in patients with different types of solid tumours were evaluated, and the correlation between these parameters and tumour stage, changes in known characteristic tumour markers, imaging findings, and the changes in haemostatic parameters which occur with different types of therapeutic intervention were assessed. The markers of hypercoagulability were monitored from the time of initial presentation and during each planned visit when the patients received the due cycle of treatment. Our main focus was on specific abnormalities or changes of the haemostatic parameters in this group of patients with different types of solid malignancies. And, because it is not possible to accurately predict those with cancer who will develop thrombosis, the results of this prospective study can be used as additional clinical evidence to recommend routine thrombo-prophylaxis is cancer patients. It is very important to mention that the magnitude of risk for development of venous thromboembolic complication for a given anti-tumour therapy is sufficiently great, and the thromboprophylaxis method is safe and effective.

We studied the prognostic values of F1+2, D-dimer, and natural inhibitors of abnormal coagulation in this group of patients with solid tumours, however, no convincing data have thus far identified one of these hypercoagulability markers as a reliable prognostic marker, except F1+2 which has been shown to correlate with advancing disease and tumour burden. Parallel with the haemostatic parameters, we also measured the characteristic tumour markers; in all cases, there was a direct correlation between changes in the haemostatic parameters, radiological imaging findings, and tumour markers (e.g., rise in D-dimer or F1+2 was associated with a drop in AT, PC, and PS, which was directly correlated with a rise in characteristic tumour markers and a progression of the malignant diseases in radiological imaging findings).

In cases of breast and ovarian cancers (12+2 patients), data on the plasma levels of D-dimer, F1+2, PC, PS, and AT illustrated excellent correlation with the tumour volumes, and tumour markers CA15.3, CA125, respectively. The correlation between haemostatic parameters, radiological findings, tumour stage, and the characteristic tumour markers were analyzed. It was clearly demonstrated that consumption of natural inhibitors of abnormal coagulation (AT, PC, PS) was significantly reduced (their levels were improved or normalized after start of treatment) with commencement of different

therapeutic interventions. In parallel, the level of D-dimer and F1+2 as markers of activated clotting cascade was markedly decreased. These changes in haemostatic system were associated with regression of tumour volume and size which were assessed by characteristic tumour markers and imaging studies, respectively. In 14 patients with breast and ovarian cancer, the improvement in haemostatic abnormalities and regression of tumour size were durable in more than 90% of cases with a follow-up period reaching 55 months.

In 16 patients with colorectal carcinoma (CRC), similar findings were observed. The levels of naturally occurring anticoagulants were almost normalized in all cases shortly after surgical resection and start of systemic chemotherapy and subsequently the values of hypercoagulable parameters were significantly reduced as a result of different therapeutic interventions. And, all the 16 patients with CRC are alive 45-50 months after the end of their treatments.

In case of patients with gastric and lung cancer, similar response was documented at the initial presentation and start of therapy both in term of improvement of haemostatic abnormalities and that of tumor size (regression of tumour size), but all those responses were of short duration. Our explanation for this short response is that; patients with gastric and lung cancer usually present and diagnosed when the primary tumour is of large size, and it had already disseminated. The improvement of haemostatic parameters was more prominent in patients with non-small-cell lung cancer compare to those with small-cell lung.

The results of changes in haemostatic parameters from this small cohort of patients in our study are in agreement with the results of previously published multiple clinical trials. It is worth mentioning, that this direct correlation between changes in haemostatic system and response of tumour to different therapeutic interventions is mostly observed in patients with adenocarcinomatous type of cancer. The tumour markers which were used in assessment of response in our study were those which are recommended by different guidelines of assessment of clinical response in patients with solid tumours. These tumour markers are not tumour-specific with exception of those in ovarian, breast, and prostate cancers and to a lesser extent in colorectal carcinoma where they can be (are) used as a useful tool for both diagnosis and follow up of these patients and also to assess

the efficacy of the treatment.

Additionally, the extent of hypercoagulability parameters which were evaluated in this cohort of patients reflect a broad spectrum combination of known markers of coagulation cascade activation and each of these markers was used separately in previous studies and reports.

To the best of our knowledge, so far there has not been any report or study in which such a broad combinations of different hypercoagulable parameters have been used. In addition, the frequency of measurements of haemostatic parameters was very high and all these values were closely monitored and correlated with those of imaging findings and characteristic tumour markers plus the very long duration of follow-up.

Finally, we attempted to describe the current theory about the pathophysiology of the hypercoagulable status in cancer patients, and we also tried to discuss whether or not to screen elder patients (patients above age of 45 years) with idiopathic deep venous thrombosis for an underlying malignancy, and whether this would be potentially beneficial to patients and to the ongoing arguments regarding economic background for prophylactic and therapeutic strategies in cancer patients.

We hope that a better and more scientific understanding of these mechanisms (to be explored by further randomized clinical trials) will ultimately lead to the development of more targeted treatments to prevent and to treat thromboembolic complications in cancer patients. Effective and safe antithrombotic therapy- the mainstream in prophylaxis and treatment of thromboembolism- remains very challenging clinical task in cancer patients- a population with high rate of treatment failure, haemorrhagic and thromboembolic complications recurrences and relapses. We also hope that guidelines for antithrombotic treatment in cancer patients may also have a positive effect on the process of tumour growth and metastasis. Based on the findings from this small cohort of patients, we may conclude that antithrombotic therapy might interfere with various processes involved in cancer development, growth and dissemination.

These results might be useful as basis for future larger scale trials in which additional markers of hypercoagulable status will be evaluated in order to identify the most sensitive marker with highest prognostic impact on patients' survival. The significant finding of the

study in which a direct correlation between hypercoagulability markers and tumour stage was confirmed, can be further assessed in future trials to compare the efficacy of different doses of low molecular weight heparins and to determine the effect of LMWHs on tumour progression. In cancer patients with good prognosis and longer life expectancy, the treatment with LMWHs could result in survival benefit.

I would like to take this opportunity to thank all those who helped me completing this work over the past 5-6 years and in particularly my family, Professor Shaker A. Mousa, Professor Hajna Losonczy, friends and colleagues. I sincerely thank them all and I wish them all the success they richly deserve. I also thank my family for being there for me whenever I needed them and in all circumstances.

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